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PII: S1466-8564(15)00062-4
DOI: doi: 10.1016/j.ifset.2015.03.013
Reference: INNFOO 1297

To appear in: *Innovative Food Science and Emerging Technologies*

Received date: 13 October 2014
Revised date: 19 February 2015
Accepted date: 12 March 2015

Please cite this article as: Tan, X., Zhang, B., Chen, L., Li, X., Li, L. & Xie, F., Effect of planetary ball-milling on multi-scale structures and pasting properties of waxy and high-amylose cornstarches, *Innovative Food Science and Emerging Technologies* (2015), doi: 10.1016/j.ifset.2015.03.013

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Effect of planetary ball-milling on multi-scale structures and pasting properties of waxy and high-amylose cornstarches

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Abstract: Waxy and high-amylose cornstarches were mechanically modified and the effects of planetary ball-milling treatment on the multi-scale structures and pasting properties of these cornstarches were investigated. The ball-milling could hardly change the structures and properties of high-amylose cornstarch but result in distinct changes to that of waxy cornstarch. With the thicker semi-crystalline lamellae, larger crystalline amylopectin lamellae, thinner amorphous amylopectin lamellae and more structural rigidity amylose amorphous background region, high-amylose cornstarch showed high resistance to the mechanical disruption during the planetary ball-milling treatment. Consistent with the structural changes, the paste properties of high-amylose starch has negligible changes but the treated waxy cornstarch showed a reduced pasting temperature and paste viscosity, increased pasting stability, and a reduced tendency to retrogradation. The results suggest that planetary ball-milling could be a potential physical method to obtain starch products with relatively low viscosity at high concentration and enhanced pasting stability.

Keywords: Planetary ball-milling; Cornstarch; Amylose/amylopectin ratio; Structure; Pasting properties

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1. Introduction

Starch as a natural polysaccharide has been widely applied in foods and non-food products. Since the starch structure plays a key role in determining its properties (Sandhu & Singh, 2007), it is highly important to ensure a desirable structure and thus properties to be achieved for specific applications. However, this has always be challenging regarding the inherent complex structure of native starch, which greatly limits the expansion of starch applications. Regarding this, various techniques involving chemical, physical and enzymatic methods are useful in improving the functional and other physicochemical properties of starch such as pasting properties and gelatinization behaviors. Besides, in recent years, physical techniques for starch modification (e.g., heat-moisture, ultrasound, microwave, high-pressure and ball-milling) have attracted great attention due to their advantages such as increased safety and reduced waste-generation (Blaszczak et al., 2007; Szepes et al., 2005; Zhu, Li, Chen, & Li, 2012; Zhang, Zhao, Li, Zhang, Li, Xie, & Chen, 2014; Huang et al., 2008).

Ball-milling has been reported as an eco-friendly and cost-effective physical technique which could regulate the starch structure and therefore modify the physicochemical properties of starch and cereal flour (Liu, Ma, Yu, Shi, & Xue, 2011; Loubes & Tolaba, 2014). Ball-milling normally can provide a mechanochemical effect on the characteristics of materials, through combined friction, collision and shear resulting from the grinding balls and the container wall. As reported earlier, the granule morphology, granule size distribution, crystallinity, molecular weight, amylose/amylopectin ratio of starch could be significantly modified by ball-milling, together with the resultant changes in the starch properties including solubility, digestibility, pasting properties, and rheological properties (Tamaki et al., 1998; Huang et al., 2008; Kim et al., 2001; Liu, Ma, Yu, Shi, & Xue, 2011).

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Therefore, ball-milling shows potentials as a physical approach to modulate the starch structure and functionalities (e.g., reduced crystallinity and increased solubility), for expanding the applications of starch (Liu, Ma, Yu, Shi, & Xue, 2011).

As a heterogeneous material, starch is normally a mixture of two biopolymers, i.e., 10–30% amylose, a mostly linear 1,4-*α*-D-glucan with a small number of long branches, and 90–70% amylopectin, mainly1,4-*α*-D-glucan but having a large number of 1,6-*α* linkages at the branch points (Karim, Norziah, & Seow, 2000; Zhang, Zhao, Li, Zhang, Li, Xie, & Chen, 2014). In addition, high-amylose starch can possess amylose content up to 85%, while waxy starch may contain 100% amylopectin after genetic modification (Liu, Halley, & Gilbert, 2010). These two biopolymers form the hierarchical structure of starch which is organized on multi-length scales, from different supramolecular structures (whole granule, growth rings, semi-crystalline lamellae, and crystalline structure) to the molecular structure (chain characteristics) (Oates, 1997). Due to the different amylose/amylopectin ratio, waxy, regular and high-amylose starches often display prominent differences in their hierarchical structure. In particular, while waxy and regular starches have a large amount of A-type crystallites, high-amylose starch predominantly displays a B-type crystalline structure. It is noteworthy that starch with a higher amylose content is less susceptible to various physicochemical treatments such as hydrothermal treatment, despite its lower crystallinity (Liu, Yu, Xie, & Chen, 2006; Zhang, Zhao, Li, Li, Xie, & Chen, 2014). Therefore, to improve the functional properties (e.g., pasting properties) of starch by ball-milling, it is extremely important to understand the effects of ball-milling on the hierarchical structure and properties of starches with different amylose/amylopectin ratios.

Compared with other conventional milling methods, planetary ball-milling can result in more

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apparent alteration to starch characteristics and thus can be considered as a more suitable method for starch modification. It has been reported that planetary ball-milling is capable to reduce starch crystallinity and double-helices (Liu, Ma, Yu, Shi, & Xue, 2011). However, the changes induced by this method in the starch multi-scale structures (especially including, on the nano-scale, semi-crystalline lamellae), and in its functionalities such as pasting properties, have not been well understood. This is especially true when starches with different amylose/amylopectin ratios are involved.

In present work, waxy and high-amylose (Gelose 80, or G80) cornstarches were selected and treated by a planetary ball mill for different times. By comparing starch samples without and with the planetary ball-milling treatment, the related changes in the granule morphology, granule size distribution, semi-crystalline lamellae, crystalline structure and molecular structure, as well as the pasting properties of waxy and high-amylose cornstarches were explored.

2. Materials and methods

2.1. Materials

Waxy cornstarch (the amylose/amylopectin ratio, 0/100) was obtained from Lihua Starch Industry Co., Ltd. (Qinhuangdao, China), and a high-amylose cornstarch, Gelose 80 (G80) (the amylose/amylopectin ratio, 80/20) was supplied by Penford (Australia). The moisture content (MC), (about 10%) of each sample was determined using a moisture analyzer (MA35, Sartorius Stedim Biotech GmbH, Germany). Anhydrous ethanol, in reagent grade, was supplied by Nanjing Chemical Reagents Co., Ltd. (Nanjing, China).

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2.2. Planetary ball-milling treatment

A QM-BP planetary ball mill (Nanda, Nanjing, China) with four ceramic milling cylinders (100 mL) were used. About 15 g of starch, and zirconia balls (a mixture of balls with diameters of 2, 5 and 10 mm) of which the weight was three times that of starch, were placed into each ceramic container, filling about 1/3 capacity of the container, followed by addition of 12 mL of anhydrous ethanol. The cylindrical container was tumbled at a rotation speed of 1032 rpm (the ratio of rotation/revolution speed, 2/1) for 4, 8, 15 or 20 h. After treatment, the sample was collected after removing the balls and ethanol (by evaporation), and then was sealed for further analyses. In the following discussion, the code typically as "Waxy-0h" is used, in which "Waxy" represents the type of cornstarch and "0h" indicates the ball-milling treatment time.

2.3. Scanning electron microscopy (SEM)

Granule morphology was observed using an EVO18 scanning electron microscope (ZEISS, Germany), operated at 10.0 kV. All the samples were coated with a thin gold film before the microscopic observation.

2.4. Laser diffraction analysis

Granule size distribution was analyzed by a Malvern Mastersizer 2000 laser-diffraction analyzer (Version 5.22, Malvern, UK) using a 1000 mL flow-through reservoir. Each ball-milled starch sample was added to the reservoir and fully dispersed in anhydrous ethanol until an obscuration value between 12% and 17% was achieved. The pump speed was set at 2050 r/min. The refractive index of the starch samples and the dispersing reagent ethanol was 1.54 and 1.36, respectively.

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Volume size distribution between 0.10 and 104.71μm was recorded for all samples. All the results are reported as the averages of three replicates.

2.5. Small-angle X-ray scattering (SAXS)

SAXS experiments were performed on a SAXSess small-angle X-ray scattering system (Anton-Paar, Austria) equipped with a PW3830 X-ray generator (PANalytical), operated at 50 mA and 40 kV, using Cu-Kα radiation with a wavelength of 0.1542 nm as the X-ray source. The samples (ca. 60% MC) used for the SAXS measurement were prepared by premixing the starches with added water in glass vials and were equilibrated at 20 °C for 24 h before the analysis. Each sample was placed in a paste sample cell and was exposed at the incident X-ray monochromatic beam for 5 min. The data, recorded using an image plate, were collected by the IP Reader software with a PerkinElmer storage phosphor system. All data were normalized, and the background intensity and smeared intensity were removed using the SAXSquant 3.0 software for further analysis.

2.6. Light microscopy

Both ordinary and polarized light micrographs were recorded using a light microscope (Axioskop 40 Pol/40APol, ZEISS, Oberkochen, Germany) equipped with a camera (PowerShot G5, Canon, Tokyo, Japan). The magnification was set at 500 (50×10). Each sample was dispersed as 10 mg of the starch in 1 mL of distilled water in a glass vial. Then, a drop of the starch suspension was transferred onto a slide, covered by a cover slip.

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2.7. X-ray diffraction (XRD)

X-ray diffraction patterns of the starch samples were measured using an Xpert PRO diffractometer (PANalytical, Netherlands), operated at 40 mA and 40 kV, using Cu-Kα radiation with a wavelength of 0.1542 nm as the X-ray source. The diffraction angle (2θ) scanning was from 5° to 40° with a scanning speed of 10°/min and a scanning step of 0.033°. The MC of each sample was about 10%.

2.8. Fourier transform Raman (FT-Raman) spectrometry

FT-Raman spectra were recorded on a Nicolet iS50 instrument (ThermoFisher, USA) with a near-infrared YAG laser with a wavelength of 1064 nm. The laser was focused on the sample at a spot of ca. 1 mm in diameter with a power of 400 mW. 1024 scans were used for each spectrum in the wavenumber range of 4000 to 300 cm^{-1} with a resolution of 8 cm^{-1}. In present work, the direct comparison of the spectrum intensities of different samples was reliable, as the layer thickness and spot laser size were practically identical for all samples.

2.9. Brabender viscoamylograph profiles

Pasting properties were analyzed on a BrabenderMicro Viscoamylograph machine (Brabender OHG, Germany). 100 g of 6.0% (w/w) starch suspensions were stirred at a paddle speed of 250 rpm. Each starch suspension was heated from 30 to 95 °C at 1.5 °C/min, held at 95 °C for 30 min, cooled from 95 to 50 °C at 1.5 °C/min, and held at 50 °C for 30 min. The data were processed and obtained using the Viscograph Data Correlation software.

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2.10. Statistical analysis

The experiments were conducted in triple (n=3) and data analyzed with SPSS 20.0 statistical software. Analysis of variance (ANOVA), followed by the Duncan's multiple-range test. The significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Granule morphology

Fig. 1 shows the SEM micrographs of waxy and G80 cornstarches after the planetary ball-milling treatment for different times. It can be seen that waxy cornstarches displayed more pronounced changes in their granule morphology after the treatment, compared with G80 cornstarch. Native waxy cornstarch consisted of large oval and polyhedral granules having slightly rough surface. As the time increased up to 15 h, waxy cornstarch granules were gradually broken into small fragments with rougher surface than native granules, resulting from the mechanochemical effect of planetary ball-milling. Nevertheless, a further increase in time (up to 20 h) did not cause a substantial change in the granule morphology of waxy cornstarch. This suggests that "grinding equilibration" existed for waxy cornstarch at a ball-milling treatment time of about15 h (Liu, Ma, Yu, Shi, & Xue, 2011). On the other hand, the treatment for up to 20 h only caused negligible changes to the granule morphology of G80 cornstarch, with a slight increase in the surface roughness. This indicates G80 cornstarch had greater resistance to the mechanical force during planetary ball-milling than waxy cornstarch. This result is in agreement with the fact that starch with higher amylose content often has stronger resistance to various physicochemical treatments (Zhang, Zhao, Li, Li, Xie, & Chen, 2014).

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3.2. Granule size distribution

Evolutions of granule volume size distributions of waxy and G80 cornstarches during the planetary ball-milling treatment are shown in Fig. 2a and b, and the related parameters are summarized in Table 1. It can be seen that both cornstarches after the treatment presented a decrease in their granule size, as indicated by a shift of the size distribution profile towards a smaller particle size range. And this trend was more obvious when the amylopectin content was higher (i.e., waxy cornstarch). This is in good agreement with the results from SEM where greater destruction to the granule morphology of waxy cornstarch could be observed. From Table 1, it is seen that $d_{(0.5)}$ (the diameter value less than which 50% of the overall granules have) of waxy cornstarch reduced from 23.7 to 4.6 μm and G80 cornstarch showed a less significant decrease in $d_{(0.5)}$ (from 12.5 to 8.3 μm). In addition, grinding equilibration was also observed for G80 cornstarch (at about 4 h) because the granule size distributions are very similar for the G80 cornstarch planetary ball-milling treated between 4h and 20h. Moreover, waxy cornstarch showed a slight decrease in the proportion of fragments with the particle size between 0 and 1 μm. This suggests that the occurrence of re-aggregation (agglomeration) of small fragments of waxy cornstarch during the planetary ball-milling treatment. Combining the results of Fig. 2 and Table 1 it can be seen that the larger waxy cornstarch granules can be easier to damage by the planetary ball-milling treatment. Since G80 cornstarch after the treatment for 4 h had larger granules than that of waxy cornstarch counterpart, the larger granule size should not be a predominant determinant for reducing the resistance of starch to planetary ball-milling. In order to reveal the effect of the difference in multi-scale structure and the structural changes during the planetary ball-milling treatment on the starch pasting properties of these two cornstarches, the two starch samples after planetary ball-milling treatment were further

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investigated.

3.3. Lamellar structure

The semi-crystalline lamellae, i.e., alternating crystalline and amorphous lamellae, of starch are normally studied by SAXS (Suzuki et al., 1997; Zhang, Zhao, Li, Li, Xie, & Chen, 2014). Based on the Woolf-Bragg's equation: $d = 2\pi/q$, the average thickness (d) of semi-crystalline lamellae can be calculated using the q position of a scattering peak at around 0.6 nm^{-1} (Vermeylen, Goderis, & Delcour, 2006). As shown in Fig. 2c and d, native waxy and G80 cornstarches possessed a scattering peak at 0.6270 and 0.5929 nm^{-1}, corresponding to a d value of 10.01 and 10.59 nm, respectively (cf. Table 1). This indicates that the semi-crystalline lamellae of the native waxy cornstarch were thinner than that of G80 cornstarch. After the planetary ball-milling treatment, the semi-crystalline lamellae thickness of waxy cornstarch gradually decreased from 10.01 to 9.53 nm, and even disappeared when the time was ≥15 h. In contrast, very small changes occurred to the thickness of semi-crystalline lamellae of G80 cornstarch after the treatment.

In general, the flawless semi-crystalline with a high degree of ordering can lead to the appearance of a SAXS scattering peak with fine visibility at around 0.6 nm^{-1}. As seen from Fig. 2c and d, the planetary ball-milling treatment decreased the visibility of peak at around 0.6 nm^{-1}, suggesting structural disorganization with a reduction in the perfection and in ordering degree of the semi-crystalline structure. Similar to the results of granule morphology and granule size distribution, the semi-crystalline of waxy cornstarch underwent a more prominent destruction than that of G80 cornstarch, although the native waxy cornstarch had a more visible SAXS peak at around 0.6 nm^{-1}.

Besides the d value, other two parameters of a theoretical model for the semi-crystalline

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lamellae in the starch granule can be obtained from the SAXS data (Cameron & Donald, 1993a, 1993b; Zhang, Li, Liu, Xie, & Chen, 2013), i.e., $\Delta\rho = \rho_c - \rho_a$ (where ρ_c and ρ_a are the electron densities of the crystalline and amorphous regions in the semi-crystalline lamellae, respectively), the difference in electron density between the crystalline and amorphous lamellae; $\Delta\rho_u = \rho_u - \rho_a$ (where ρ_u is the electron density of the amorphous background), the difference in election density between the amorphous lamellae and the amorphous background. The major effect of increasing $\Delta\rho$ is to increase the overall intensity including the peak intensity, and $\Delta\rho_u$ has simultaneous effects of raising the low-angle intensity and lowering the definition of the peak without changing the peak position (Cameron & Donald, 1992). From Fig. 2c, a decrease in the definition of the peak (around 0.6 nm^{-1}) was observed for waxy cornstarch with the time increasing up to 8 h, together with an increase in the scattering intensity at low q values. This indicates a decreased $\Delta\rho$ and an increased $\Delta\rho_u$ resulting from the greatest destruction to the crystalline lamellae, the intermediate destruction to the amorphous lamellae, and the weakest destruction to the amorphous background. Nevertheless, the planetary ball-milling treatment for even longer times (15 and 20 h) would make the semi-crystalline lamellae of waxy cornstarch undetectable, presumably due to a higher degree of disorganization in the semi-crystalline lamellae. Unlike waxy cornstarch, G80 cornstarch after the planetary ball-milling treatment presented moderate changes in the SAXS patterns, i.e., an increase in the overall intensity at low q values, but a decrease in the definition of the scattering peak (which became broader). This indicates that $\Delta\rho$ and $\Delta\rho_u$ of G80 cornstarch could be simultaneously increased by planetary ball-milling, due to the greatest destruction to the amorphous lamellae, the intermediate destruction to the amorphous background materials, and the weakest destruction to the crystalline lamellae.

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According to the understanding of starch granule structures, compared with G80 cornstarch, waxy cornstarch contains only amylopectin with more short A-chains (DP 6–12) (Hanashiro, Abe, & Hizukuri, 1996; Jane, Wong, & McPherson, 1997), and thus has thinner crystalline amylopectin lamellae (Hizukuri, Kaneko, & Takeda, 1983) but thicker amorphous amylopectin lamellae. G80 cornstarch has ca. 80% amylose which presents in the amorphous background region, imparting a 'harder' structure to such region, while ca. 20% amylopectin in G80 cornstarch forms larger crystalline amylopectin lamellae and thinner amorphous amylopectin lamellae. Consistent with these findings, the different susceptibilities of starch granules to mechanical disruption for G80 and waxy cornstarches should be related to their nano-scale structural difference. With thicker semi-crystalline lamellae, larger crystalline amylopectin lamellae, thinner amorphous amylopectin lamellae and a larger amount of amylose amorphous background region with structural rigidity, G80 cornstarch showed higher resistance to the mechanical disruption during the planetary ball-milling treatment.

3.4. Crystalline structure

The starch granule is a semi-crystalline system consisting of crystalline and amorphous regions, and when it is under polarized light, a polarization cross (birefringence) can be seen. Fig. 3a presents the ordinary and polarized light micrographs of waxy and G80 cornstarches before and after the planetary ball-milling treatment for different times. Similar evolutions of the granule morphology as those from the SEM micrographs (Section 3.1) could be observed. Moreover, as the intensity of birefringence is related to the granule size, degree of crystallinity and microcrystalline orientation, the destruction to the crystalline structure of waxy cornstarch by planetary ball-milling could conceivably result in reduced birefringence intensity. However, for the G80 cornstarch there were

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negligible changes in the birefringence. This indicates that planetary ball-milling treatment could hardly destruct the crystalline lamellae of G80 cornstarch.

For further examining the crystalline structural changes, Fig. 3b and c show the XRD patterns of waxy and G80 cornstarches after the planetary ball-milling treatment for different times. It can be seen that the crystalline parts of starch showed sharp peaks but the rest amorphous parts presented dispersive patterns (Gernat, Tadosta, & Damaschun, 1990). Apparently, the native waxy cornstarch displayed a typical A-type crystalline structure with main diffraction peaks at 2θ of around 15°, 17°, 18° and 23°, whereas native G80 cornstarch displayed a B-type crystalline structure. It is noted that the results from Fig. 3b and c correspond well to the results from the polarized light micrographs, as after the treatment for different times, a substantial reduction in the intensities of A-type crystalline peaks occurred for waxy cornstarch, whereas G80 cornstarch only showed slightly changed XRD patterns.

As shown by the previous study (Jane, Wong, & McPherson, 1997; Bayer, Cagiao, & Calleja, 2006), while the crystallinity of native starch granules is mainly associated with the ordered three-dimensional structure of amylopectin. A-type crystalline starch is likely to contain the α-1,6 branch linkages, which are more scattered and are located within both the crystalline region and the amorphous region, while most of the branch linkages in the B-type crystalline starch are clustered in the amorphous region. The scattered branch points located inside the crystalline region leads to "weak points" in the A-type crystalline starch granules. Thus, the A-type crystalline lamellae region was easier to damage. This is in good agreement with the results from SAXS test that a decreased $\Delta\rho$ and an increased $\Delta\rho_u$ resulting from the greatest destruction to the crystalline lamellae for waxy cornstarch. Furthermore, the A-type crystalline structure has a monoclinic crystal unit with 8

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inter-helical water molecules, whereas the B-type crystalline structure has a more open packing of helices with 36 inter-helical water molecules in each hexagonal crystal unit. Hence, during planetary ball-milling, the larger amount of inter-helical water in the B-type crystallites could contribute to more hydrogen bonds and a better hydrogen bonding network, making B-type crystalline lamellae of G80 having greater resistance to the mechanical force during planetary ball-milling which has been observed by the SAXS test that $\Delta\rho$ and $\Delta\rho_u$ of G80 cornstarch simultaneously increased because of the weakest destruction to the crystalline lamellae. On the other hand, our recent findings (Zhang, Zhao, Li, Li, Xie, & Chen, 2014) disclosed that the amylose molecules could act as the backbones of the aggregation structures to provide resistance to the physical treatment of heat-moisture. The B-type crystalline starch has been confirmed to have a higher ordering degree of surface structure, acting as a factor to enhance the resistance to planetary ball-milling for the B-type G80 cornstarch (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). On the basis of these findings, it is reasonable to infer that under a same milling condition, planetary ball-milling would more powerfully alter the organization of waxy cornstarch crystallites, and thus a more prominent amorphisation could be observed for waxy cornstarch.

3.5. Molecular chain characteristics

FT-Raman technique has been reported to be used for investigating the morphology and structures of polymers (Qin, & Kean, 1998). The FT-Raman spectra of waxy and G80 cornstarches before the planetary ball-milling treatment are shown in Fig. 4a, which shows a similar pattern for native waxy and G80 cornstarches. Specifically, the band at 2910 cm^{-1} was attributed to the O-H and C-H stretching vibrations, while the band 480 cm^{-1} was ascribed to the skeletal modes involving the

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(C-O-C) ring and δ(C-C-O). Also there were other characteristic bands for polysaccharides, e.g., the peak at 860 cm^{-1} originating from $V_s(C_1\text{-}O\text{-}C_5)$ ring mode and C_1-H bending of α-configuration, the peak at 940 cm^{-1} assigned to $V_s(C_1\text{-}O\text{-}C_{4'})$ of α-1,4-glycosidic linkage, the peak at 1390 cm^{-1} due to δ(C-OH), C-H bending and CH_2 scissoring vibrations, and the peak at 1460 cm^{-1} resulting from δ(CH_2) twisting and C-H bending (Mutungi et al., 2012; Łabanowska et al., 2013).

After planetary ball-milling, the FT-Raman intensity for waxy cornstarch in the range of 960 to 800 cm^{-1} was reduced gradually as the time increased (see Fig. 4b). It can be inferred that the breakage of waxy cornstarch glycosidic linkage occurred, since the peak intensity at 940 cm^{-1} related to C_1 and C_4 was reduced. And the glucose units of waxy cornstarch were substantially degraded into short fragments by the planetary ball-milling treatment, as indicated by the shift and decrease of the peak at 860 cm^{-1}. On the other hand, G80 cornstarch showed negligible changes in the FT-Roman spectra (see Fig. 4b), indicating no apparent alteration to its molecular chains. As discussed above, compared with waxy cornstarch, G80 cornstarch possessed more robust supramolecular structures (semi-crystalline lamellae, and crystalline structure, etc.), which had greater resistance to the mechanical force of planetary ball-milling. This robustness could minimize the destruction to the molecular chains.

3.6. Pasting properties

Fig. 5a shows the pasting profiles of G80 corn starch samples without and with the planetary ball-milling treatment (20 h). It is widely accepted that the typical pasting profile representing the gelatinization process cannot be observed for native G80 cornstarch since the highest temperature (95 °C) during the measurement is insufficient for the melting of crystalline structure of native G80

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cornstarch (Zhang, Zhao, Li, Li, Xie, & Chen, 2014). Similar to native G80 cornstarch, the G80 cornstarch sample after the treatment for 20 h did not present a gelatinization-like pasting profile. This is consistent with the negligible changes in the hierarchical structure of G80 cornstarch after planetary ball-milling treatment.

However, from Fig. 5b and Table 3, for waxy cornstarch, a decrease in the pasting parameters was observed after the treatment for a prolonged time. This was caused by the prominent structural destruction to waxy cornstarch by the treatment. Specifically, not only could the decreased granule size (i.e., increased specific surface area) with broken surface promote the water permeability into the granules, but also the disorganization in the lamellae and crystallites could weaken the thermal stability of the supramolecular structures. As a consequence, the modified waxy cornstarch samples displayed weaker resistance to the swelling and rupture while being heated in water, resulting in a reduced pasting temperature (T_p) as shown in Table 3.

Furthermore, despite that the less ordered degree of the supramolecular structure could enhance the granule swelling, the swollen and/or broken granules had a fragile surface which could decrease the resistance to shear and therefore reduce their swelling degree. In addition, the granules became smaller after the treatment, resulting in reduced size of the fully swelled granules. Thus, a reduction in the maximum viscosity (η_{pk}) occurred for the treated waxy cornstarch samples. Nevertheless, the paste viscosity (η_{sc}) at the start of cooling showed less significant difference without and with planetary ball-milling, although the treatment induced breakage of the molecular chains which could decrease the η_{sc} value. In this way, the modified waxy cornstarch samples displayed a smaller viscosity breakdown (η_{bd}, $\eta_{pk} - \eta_{sc}$) than that of the native sample, indicating their higher paste stability at 95 °C. Again, compared with the native granules, the treated granules displayed a reduced

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swelling degree and were less intact. This made the treated granules less easy to be ruptured during the pasting process from η_{pk} to η_{sc}. This could also be the reason for the reduced viscosity breakdown.

During paste cooling, the degraded molecular chains of waxy cornstarch resulting from planetary ball-milling (as confirmed by the FT-Raman technique) were less easy for rearrangement. Therefore, the treated waxy cornstarch samples had a lower tendency to retrogradation and higher paste stability during cooling as demonstrated by the reduced η_{sb} ($\eta_{ec} - \eta_{sc}$) (cf. Table 3). The reduced tendency to retrogradation could also contribute to a more stable viscosity during paste holding at 50 °C. In addition, the pasting study showed that grinding equilibration could be achieved at 15 h, and further treatment by planetary ball-milling would not result in any further change to the pasting parameters for waxy cornstarch.

4. Conclusion

In summary, this work provides insights into the effects of planetary ball-milling on the multi-scale structures and pasting properties of cornstarch. It was found that the variation in the starch supramolecular structures by the amylose/amylopectin ratio could result in a different degree of resistance of starch to the planetary ball-milling treatment. Compared with G80 cornstarch, the waxy cornstarch was more susceptible to planetary ball-milling, as shown by more significant changes in the multi-scale structures and pasting properties. This could result from the less robust supramolecular structures of waxy cornstarch, as shown by SEM, laser diffraction analysis, SAXS, light microscopy, and XRD. Waxy cornstarch after the treatment displayed a reduced pasting temperature and paste viscosity, enhanced pasting stability at different temperatures, and a smaller

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tendency to retrogradation. This enables planetary ball-milling to be a potential physical technique to produce starch products with desired pasting behaviors, for expanding the applications of starch in foods and non-food products. The data from the current work provides not only better understanding of the effects of planetary ball-milling on the characteristics of starches with different amylose/amylopectin ratios, but also fundamental knowledge in design of planetary ball-milling processes for more accurate physical modification of starches in the future.

Acknowledgments

The authors would like to acknowledge the National Science and Technology Supporting Program Projects (2012BAD33B04, 2012BAD34B07), National Natural Science Funds of China (No. 31271824), the Ministry of Education Special R&D Funds for the Doctoral Disciplinary Stations in Universities (20120172110014), the Office of Education of Guangdong Province Science and Technology Innovation (Key) Projects in Universities (2012CXZD0006), the Ministry of Education Program for Supporting New Century Excellent Talents (NCET-12-0193) and the Fundamental Research Funds for the Central Universities (2013ZG0009).

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Table 1 Granule volume size distributions of waxy and G80 cornstarches treated by planetary ball-milling for different times [A)].

Sample	Milling Time (h)	Volume size distribution (%)							$d_{(0.5)}$
		110–90 μm	90–50 μm	50–20 μm	20–10 μm	10–4 μm	4–1 μm	1–0 μm	μm
Waxy	0	0.21±0.01	7.77±0.03	44.03±0.02 [aB)]	32.31±0.06 [a]	8.07±0.07 [d]	1.92±0.04 [e]	5.69±0.02 [e]	23.66±0.03 [a]
	4	--	--	0.06±0.01 [b]	22.82±0.02 [b]	52.47±0.04 [a]	14.63±0.01 [d]	10.02±0.01 [b]	7.84±0.01 [b]
	8	--	--	0.07±0.01 [b]	15.10±0.02 [c]	49.80±0.02 [b]	23.90±0.05 [c]	11.13±0.03 [a]	6.27±0.01 [c]
	15	--	--	--	6.80±0.03 [d]	46.39±0.02 [c]	37.75±0.01 [b]	9.06±0.01 [d]	4.66±0.01 [d]
	20	--	--	--	6.23±0.01 [e]	46.40±0.02 [c]	38.08±0.02 [a]	9.29±0.01 [c]	4.61±0.01 [e]
G80	0	--	0.69±0.01	18.29±0.02	35.60±0.01 [a]	29.24±0.02 [e]	4.86±0.01 [d]	11.32±0.02 [a]	12.51±0.02 [a]
	4	--	--	--	26.18±0.01 [b]	56.76±0.03 [d]	7.70±0.01 [c]	9.36±0.02 [d]	8.51±0.01 [b]
	8	--	--	--	25.00±0.01 [c]	57.57±0.01 [b]	7.91±0.02 [b]	9.52±0.02 [c]	8.39±0.01 [c]
	15	--	--	--	25.02±0.01 [c]	57.53±0.02 [c]	8.78±0.01 [a]	8.67±0.01 [e]	8.38±0.01 [c]
	20	--	--	--	23.81±0.01 [d]	58.39±0.03 [a]	7.92±0.01 [b]	9.88±0.01 [b]	8.26±0.00 [d]

A) 50% of the overall particles showed a size less than this value (μm).

B) Values are means ± SD (standard deviation) of three determinations (n = 3); values followed by the different uppercase letter within a column differ significantly ($P < 0.05$).

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Table 2 Lamellar structural characteristics, as measured by SAXS, of waxy and G80 cornstarches treated by planetary ball-milling for different times.

Sample	q [A)] (nm^{-1})	d [B)](nm)	Sample	q (nm^{-1})	d (nm)
Waxy-0h	0.6270±0.0034[cC)]	10.01±0.05[a]	G80-0h	0.5929±0.0014[a]	10.59±0.02[b]
Waxy-4h	0.6360±0.0019[b]	9.87±0.03[b]	G80-4h	0.5858±0.0028[b]	10.72±0.05[a]
Waxy-8h	0.6588±0.0012[a]	9.53±0.02[c]	G80-8h	0.5857±0.0031[b]	10.72±0.06[a]
Waxy-15h	--	--	G80-15h	0.5858±0.0023[b]	10.72±0.04[a]
Waxy-20h	--	--	G80-20h	0.5855±0.0014[b]	10.73±0.03[a]

A) q, the position of scattering peak.

B) d, the average thickness of semi-crystalline lamellae of starch.

C) Values are means ± SD (standard deviation) of three determinations (n = 3); values followed by the different uppercase letter within a column differ significantly ($P < 0.05$).

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Table 3 Pasting characteristics of waxy cornstarch treated by planetary ball-milling for different times

Sample	T_p (°C)	η_{pk} (BU)	η_{sh} (BU)	η_{sc} (BU)	η_{ec} (BU)	η_f (BU)	η_{bd} (BU)	η_{sb} (BU)	$\eta_f - \eta_{ec}$ (BU)
Waxy-0h	$69.8 \pm 0.2^{aA)}$	225 ± 3^{a}	121 ± 2^{a}	72 ± 2^{a}	106 ± 1^{a}	104 ± 1^{a}	153 ± 1^{a}	34 ± 1^{a}	-2 ± 1^{b}
Waxy-4h	66.9 ± 0.1^{b}	133 ± 2^{b}	85 ± 1^{b}	52 ± 1^{b}	73 ± 2^{b}	72 ± 1^{b}	81 ± 2^{b}	21 ± 1^{b}	-1 ± 1^{b}
Waxy-8h	66.2 ± 0.2^{c}	67 ± 1^{c}	56 ± 2^{c}	34 ± 1^{c}	46 ± 1^{c}	47 ± 1^{c}	33 ± 1^{c}	12 ± 1^{c}	1 ± 1^{a}
Waxy-15h	62.0 ± 0.2^{d}	53 ± 2^{d}	48 ± 2^{d}	32 ± 1^{d}	44 ± 2^{c}	46 ± 1^{d}	21 ± 3^{d}	12 ± 1^{c}	2 ± 1^{a}
Waxy-20h	61.8 ± 0.1^{d}	52 ± 2^{d}	47 ± 1^{d}	32 ± 1^{d}	44 ± 1^{c}	46 ± 1^{d}	20 ± 1^{d}	12 ± 1^{c}	2 ± 1^{a}

T_p, pasting temperature; η_{pk}, peak viscosity; η_{sh}, viscosity at start of holding (95 °C); η_{sc}, viscosity at start of cooling (95 °C); η_{ec}, viscosity at end of cooling; η_f, final viscosity; η_{bd} ($\eta_{pk} - \eta_{sc}$), breakdown viscosity; and η_{sb} ($\eta_{ec} - \eta_{sc}$), setback viscosity.

A) Values are means ± SD (standard deviation) of three determinations (n = 3); values followed by the different uppercase letter within a column differ significantly ($P < 0.05$).

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Figure captions

Fig. 1 SEM images of waxy and G80 cornstarches treated by planetary ball-milling for different times.

Fig. 2 Granule size distributions (a, b), and double-logarithmic SAXS patterns (c, d) of waxy (a, c) and G80 (b, d) cornstarches treated by planetary ball-milling for different times.

Fig. 3 Light microscopic images (a) and X-ray diffraction patterns (b, c) of waxy and G80 cornstarches treated by planetary ball-milling for different times.

Fig. 4 Raman spectra of waxy and G80 cornstarches (a, native cornstarches in the range of 4000 to 400 cm^{-1}; b and c, waxy and G80 cornstarches without and with planetary ball-milling treatment in the range of 960 to 800 cm^{-1}, respectively).

Fig. 5 Pasting profiles of G80 (a) and waxy (b) cornstarches treated by planetary ball-milling for different times.

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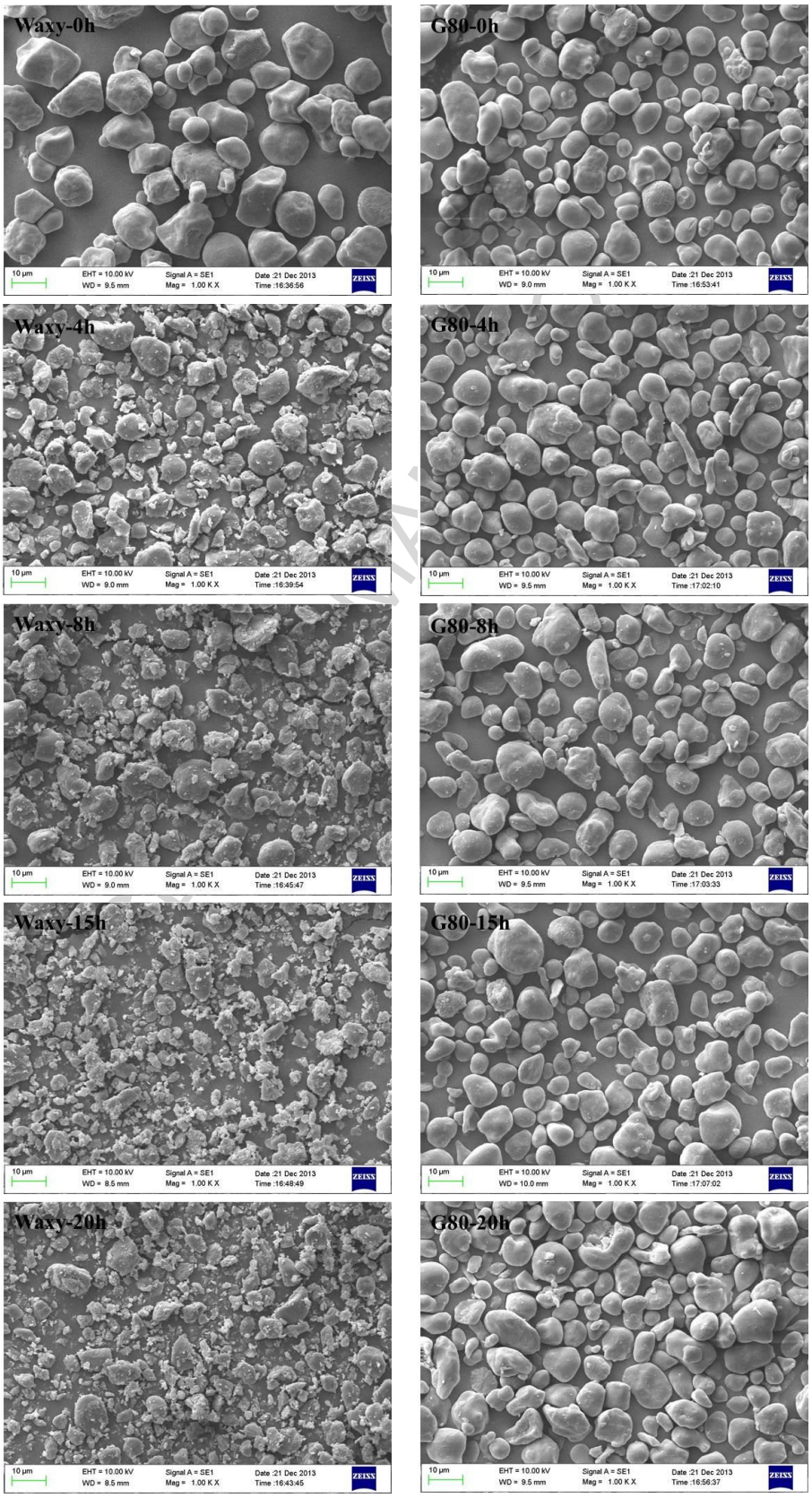

Fig. 1

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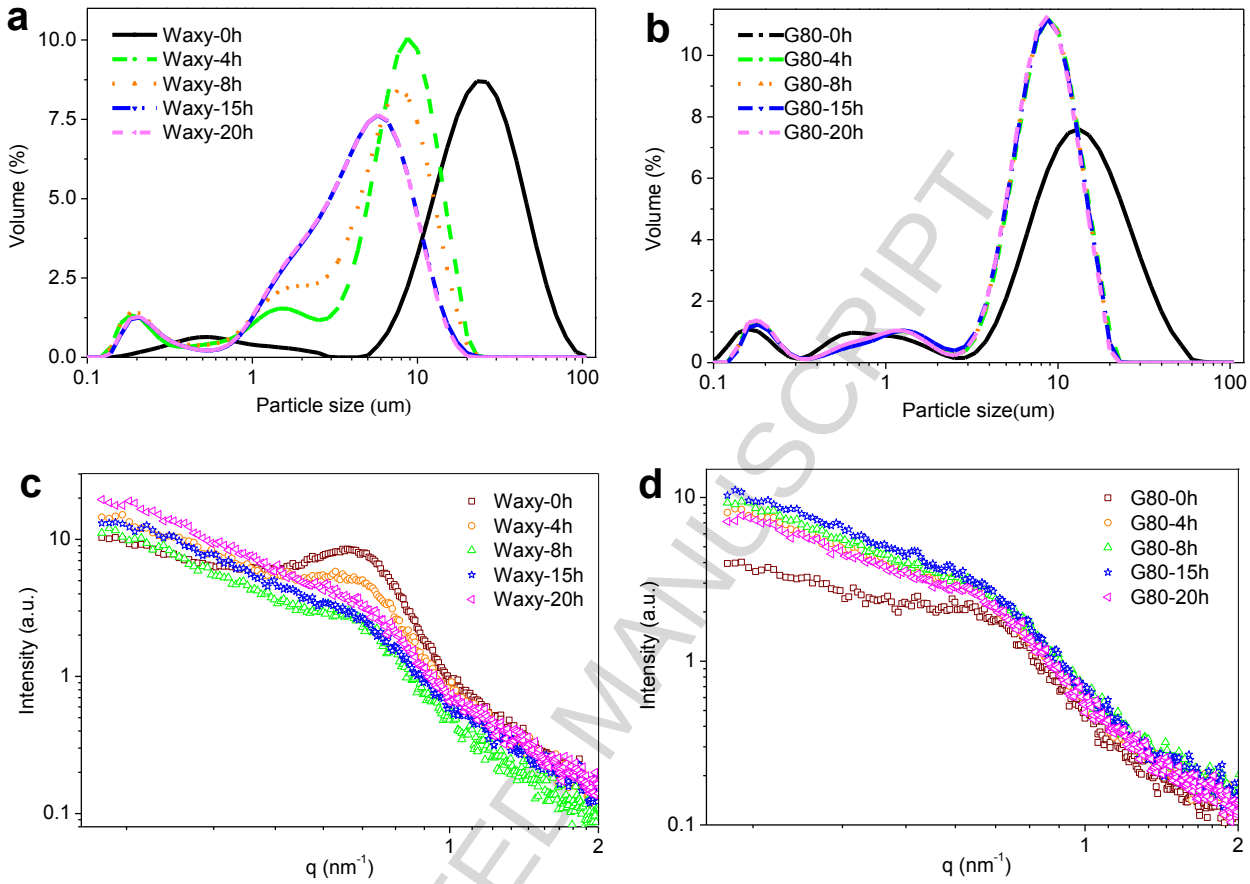

Fig. 2

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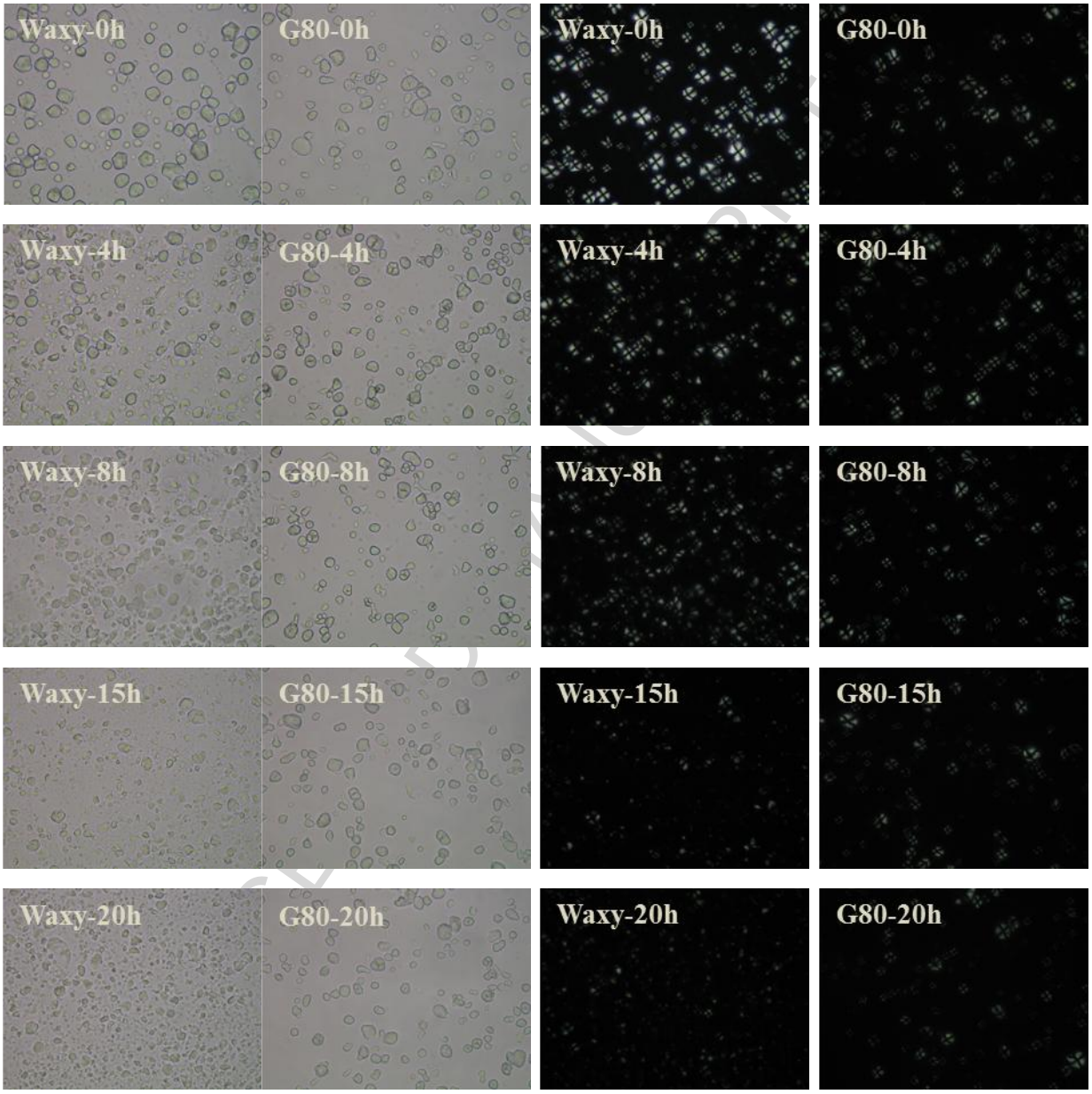
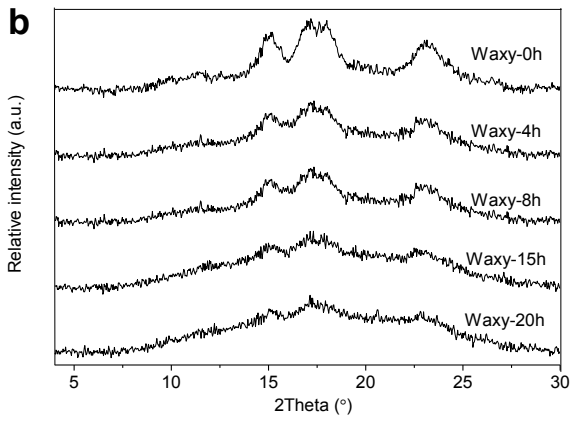
a





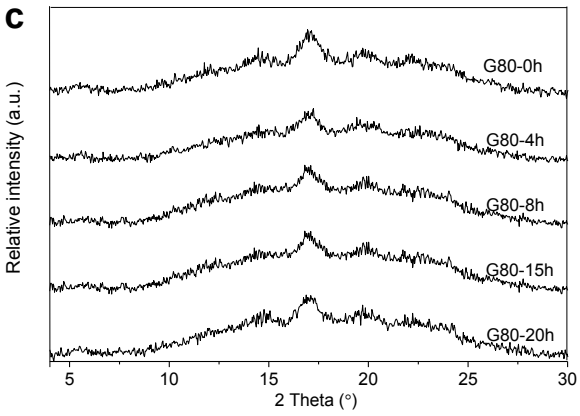


Fig. 3

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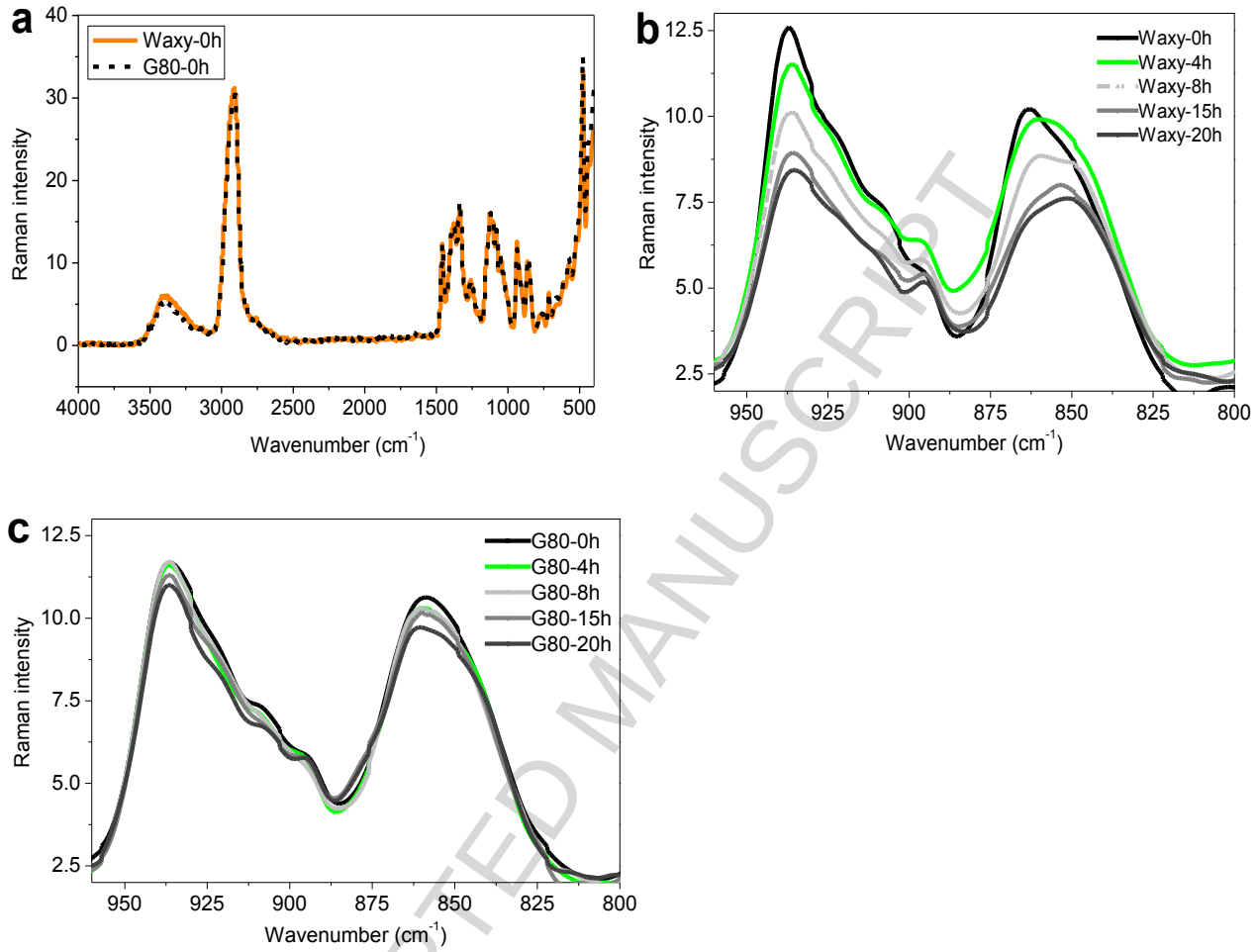

Fig. 4

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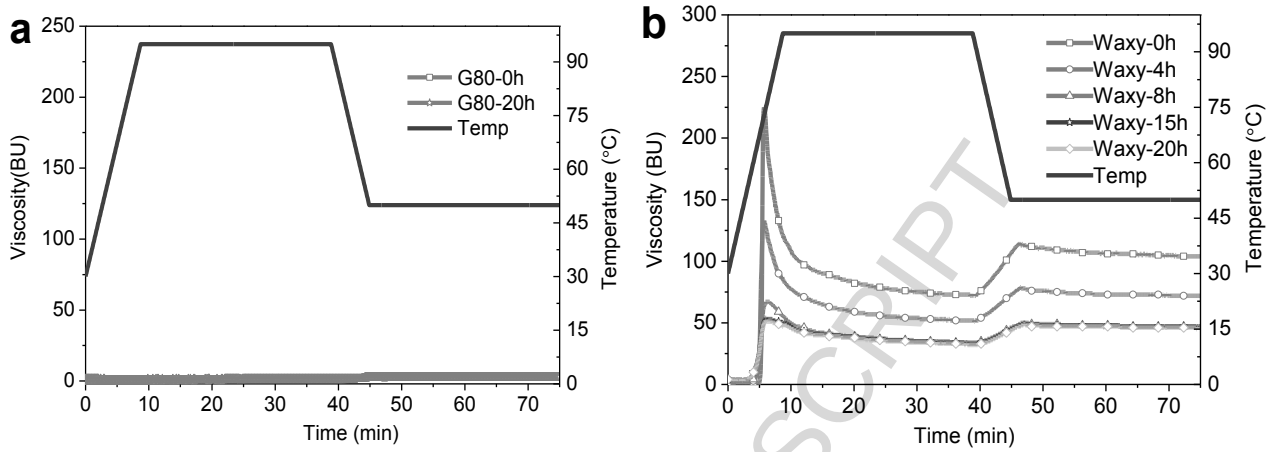

Fig. 5

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Industrial relevance:

Ball-milling is an eco-friendly and cost-effective physical technique which regulates the structure and therefore the physicochemical properties of polymers. Starch is a natural polysaccharide and has been widely used in foods and non-food products. As starch structure plays a key role in determining its properties, it is highly important to ensure a desirable structure and thus properties to be achieved for specific applications. The present study reveals that planetary ball-milling is an attractive technique to alter the multi-scale structures of starch (in particular waxy starch) and therefore its paste properties. Especially, the treatment displayed a reduced pasting temperature and paste viscosity, enhanced paste stability at different temperatures, and a smaller tendency to retrogradation, which makes starch suitable for a wide range of products such as confections, instant desserts, and canned and bottled foods. This enables planetary ball-milling to be a potential physical technique to produce starch products with desired paste behaviors and to expand the industrial applications of starch.

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Highlights

► Multi-scale structural and paste property changes after ball-milling were revealed.

► High-amylose cornstarch showed more resistance to mechanical disruption.

► Paste behaviors of waxy cornstarch could be modulated by planetary ball-milling.

► Treated waxy cornstarch showed increased paste stability and reduced retrogradation.

► Planetary ball-billing is a potential technique to regulate starch paste properties.

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